## REMARKS

This is meant to be a complete response to the Office Action mailed May 15, 2009. In the Office Action, the Examiner rejected Applicants' claims 31-37, 42, 45-51, 60 and 61 under 35 U.S.C. 112, first paragraph (written description requirement), and claims 31-37, 42, 45, 46, 48-51, 60 and 61 under 35 U.S.C. 112, first paragraph (written description requirement). Also in the Office Action, the Examiner rejected claims 31-37, 42, 45, 46, 48-51, 60 and 61 under 35 U.S.C. 103(a) as being unpatentable over US 5,482,841, in view of US 5,292,641, US 6,232,445, DiBrino et al. (Biochemistry 34(32):10130-10138 1995), and Zemmour et al. (J. Immunol. 148(6):1941-1948 1992).

Applicants' Response to the First Written Description Rejection

In the Office Action, the Examiner rejected Applicants' claims 31-37, 42, 45-51, 60 and 61 under 35 U.S.C. 112, first paragraph (written description requirement).

Applicants respectfully traverse the rejection; however, for the sake of expediting issuance of a patent from the subject application, claims 31, 35 and 61 have been amended herein to replace "at least one MHC trimolecular complex" with "an MHC trimolecular complex". Therefore, Applicants respectfully submit that claims 31-37, 42, 45-51, 60 and 61 fully comply with the written description requirement of 35 U.S.C. 112, first paragraph. Applicants respectfully request reconsideration and withdrawal of said rejection.

Applicants' Response to the Second Written Description Rejection

In the Office Action, the Examiner rejected Applicants' claims 31-37, 42, 45, 46, 48-51, 60 and 61 under 35 U.S.C. 112, first paragraph (written description requirement).

Applicants respectfully traverse the rejection; however, for the sake of expediting issuance of a patent from the subject application, claim 31 has been amended herein to replace "mRNA encodes at least one MHC heavy chain allele" with "mRNA encodes an MHC heavy chain allele". Therefore, Applicants respectfully submit that claims 31-37, 42, 45, 46, 48-51, 60 and 61 fully comply with the written description requirement of 35 U.S.C. 112, first paragraph. Applicants respectfully request reconsideration and withdrawal of said rejection.

Applicants' Response to the 35 U.S.C. 103(a) Rejection

In the Office Action, the Examiner rejected Applicants' claims 31-37, 42, 45, 46, 48-51, 60 and 61 under 35 U.S.C. 103(a) as being unpatentable over US 5,482,841, in view of US 5,292,641, US 6,232,445, DiBrino et al. (Biochemistry 34(32):10130-10138 1995), and Zemmour et al. (J. Immunol. 148(6):1941-1948 1992).

Applicants respectfully traverse the rejection for the reasons stated herein below.

The present invention, as recited in the claims, is a method for detecting the presence of anti-MHC antibodies in a sample. In the method, a pool of functionally active, recombinantly produced, truncated individual soluble MHC trimolecular complexes is obtained by the steps of isolating mRNA encoding an MHC heavy chain allele from a source, reverse transcribing the mRNA to obtain cDNA; identifying an individual MHC heavy chain allele in the cDNA; and PCR amplifying the individual MHC heavy chain allele in a locus-specific manner to produce a PCR product having the coding regions encoding cytoplasmic and transmembrane domains of the individual MHC heavy chain allele removed such that the PCR product encodes a truncated, soluble form of the individual MHC heavy chain molecule. The PCR product is then cloned into a mammalian expression vector, thereby forming a construct that encodes the individual soluble MHC heavy chain molecule, and the construct is transfected into a mammalian cell line that expresses multiple surface-bound native class I endogenous MHC molecules. The mammalian cell line is then cultured under conditions which allow for expression of the recombinant individual soluble MHC heavy chain molecule from the construct, such conditions also allowing for endogenous loading of a peptide ligand into the antigen binding groove of each individual soluble MHC heavy chain molecule in the presence of beta-2-microglobulin to form the individual soluble MHC trimolecular complexes prior to secretion of the individual soluble MHC trimolecular complexes from the cell, wherein each trimolecular complex comprises a recombinant, soluble MHC heavy chain allele, beta-2-microglobulin and endogenously loaded peptide. In addition, **each trimolecular complex of the pool of functionally active, recombinantly produced, truncated individual soluble MHC trimolecular complexes has the *same* recombinant, soluble MHC heavy chain allele. The individual,**

**soluble MHC trimolecular complexes are then purified substantially away from other proteins** and maintain the physical, functional and antigenic integrity of the native MHC trimolecular complex.

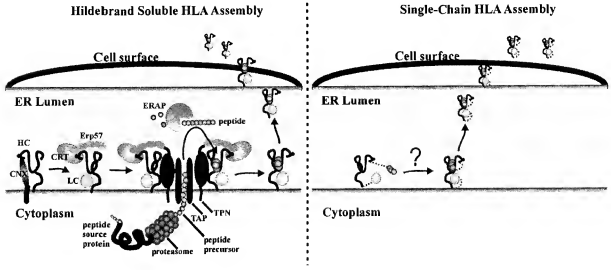
The method further includes the steps of linking an MHC trimolecular complex directly or indirectly to a substrate such that it retains the physical, functional and antigenic integrity of the native MHC trimolecular complex. A sample is reacted with the substrate/MHC trimolecular complex and washed to remove unbound portions of the sample. The substrate/MHC trimolecular complex is then reacted with means for detecting anti-MHC antibodies, and it is determined that anti-MHC antibodies specific for the individual MHC molecule are present in the sample if the means for detecting anti-MHC antibodies is positive.

The application as originally filed indicated that assays for determining the presence of antibodies or other receptors specific for alloantigens were previously known, and US Patent No. 5,482,841 was specifically cited in the application as an example of said assay (see paragraph [0010] of the application as originally filed). In addition, US 5,292,641 provides another example of prior art methods of alloantigen testing using a binding assay. However, the novelty and non-obviousness of the presently claimed invention lies not simply in the assay itself, but rather the novelty and non-obviousness of the presently claimed invention lies in the ***combination*** of a method for detecting the presence of anti-MHC antibodies in a sample with a **novel and non-obvious method of producing individual, soluble MHC trimolecular complexes,** wherein ***said complexes are used in the method*** of detecting the presence of anti-MHC antibodies.

As background, multiple methods of creating class I MHC molecules were known in the prior art; however, said methods suffered from several defects and disadvantages, as discussed in greater detail herein below. In the current application, the Applicants describe a method for the creation of soluble class I MHC trimolecular complexes that is completely different from other known methods. The main difference between prior art methods and the methods of the presently claimed invention is that the soluble class I MHC trimolecular complexes produced in the presently claimed methods are **naturally assembled within the cell** to form heterotrimers comprising the recombinantly introduced heavy chain, naturally or endogenously produced

light chain ($\beta_2$m), and naturally produced and endogenously loaded antigenic peptides. Once properly assembled by the host cell, the soluble class I MHC trimolecular complexes produced in accordance with the methods of the present invention are secreted outside of the cell, thus enabling their purification away from other proteins as well as their use in the other method steps of the presently claimed invention.

Previous technologies (such as those disclosed in US 6,232,445) have relied on mutagenesis strategies to artificially link either two components (heavy chain and $\beta_2$m) or three components (heavy chain, $\beta_2$m, and peptide) using flexible linkers. Utilizing these known technologies, at least the heavy chain and $\beta_2$m are translated together as a single polypeptide, and then folded together by the cell to attain their proper conformation. In the event a peptide is attached by a linker, this peptide typically folds "back" into the peptide-binding groove of the heavy chain. These artificial molecules can then be created in a number of cell lines, or can be used to make soluble HLA molecules composed of a single chain. The events that occur within the endoplasmic reticulum to create the folded molecule (including the chaperones involved) are unclear, however it is unlikely that the classical class I pathway is used.



The above figure represents and illustrates the different mechanisms involved in the production of the soluble class I MHC trimolecular complexes utilized in accordance with the methods of the present invention (left panel, entitled "Hildebrand Soluble HLA Assembly") and the single chain soluble class I MHC trimolecular complexes produced by others and, for example, according to U.S. Patent No. 6,232,445 to Rhode et al.

In the Rhode et al. patent, there is no demonstration that the artificially linked constructs interact with the many intracellular chaperones that are needed for proper endogenous peptide loading. In contrast, the Applicants performed experiments (as outlined in the present specification and the parent applications USSN's 10/337,161 and 10/022,066, which were incorporated by reference) that show that Applicants' truncated soluble HLA class I heavy chain traffics naturally through the cell and complexes with beta-2-microglobulin that is native to the cell; thereafter, this dimer is loaded with thousands of different endogenous and naturally produced peptides – the same process that takes place within a normal unmanipulated cell. Such trafficking is one of the main components of the Applicants' currently claimed invention -- the modified heavy chain molecule that is recombinantly inserted into the host cell interacts naturally and appropriately with the cell's chaperones (including assembly with native endogenously produced beta-2-microglobulin) and is loaded with natural and endogenously produced peptides. Applicants' methodology involves the removal of a portion of the class I heavy chain; the truncated class I heavy chain maintains its function and traffics through the cell as the native full length version, except that the truncated class I heavy chain is soluble, and is thus a secreted molecule that can be harvested. These secreted MHC trimolecular complexes can then be utilized in the claimed method for detecting antibodies specific for the individual MHC trimolecular complexes that contain the ***same*** MHC heavy chain molecule, endogenous β-2-microglobulin and endogenously loaded peptide.

Further, in nature class I MHC heavy chain molecules associate and dissociate with their light chain and peptides. The strongest binding peptides remain associated, while weaker ones rapidly dissociate. Allowing the three components of the soluble class I MHC trimolecular complexes to naturally associate/dissociate yields a more natural final product. For instance, $\beta_2$m dissociation and reassociation is used to allow peptides to be displaced from the peptide-

binding groove. By flooding the soluble class I molecules with excess $\beta_2$m, the Applicants' claimed soluble class I MHC trimolecular complexes can be used in peptide-binding assays that force peptide replacements.

In addition, another advantage of the presently claimed invention over the prior art is the fact that the MHC trimolecular complexes are recombinantly truncated so that the complexes are no longer membrane bound but rather are soluble and thus secreted from the cell, thereby greatly aiding in the ability to purify the individual, soluble MHC trimolecular complexes away from other proteins in sufficient amounts as well as retain the activity (i.e., the conformation) thereof without denaturing the complexes. Prior art references such as US 5,482,841 and DiBrino et al. utilize detergent solubilization, which has a variety of disadvantages over the presently claimed invention. The most important disadvantage is that MHC/HLA complexes purified by detergent solubilization will include a mixture of HLA molecules and thus do not provide a method for purifying individual, soluble MHC trimolecular complexes as recited by the present claims. **This disadvantage is especially true for DiBrino et al., where the Examiner has particularly pointed out that the cell line in which HLA-B*4403 was produced also expresses HLA-Cw4 as well as HLA-B35 (see Zemmour et al.). Therefore, the methods of DiBrino et al. cannot possibly purify an *individual*, soluble MHC trimolecular complex substantially away from other proteins, as the purification methods of DiBrino et al. (i.e., W6/32 immunoaffinity chromatography) do not distinguish between the *three different* HLA molecules produced by the methods taught therein.** Therefore, the methods of DiBrino et al., as modified by Zemmour et al., do not teach nor suggest a method that utilizes a pool of soluble MHC trimolecular complexes that have been **purified substantially away from other proteins**, wherein each trimolecular complex present in the pool has the same individual MHC heavy chain molecule. It is clearly evident to a person having ordinary skill in the art that any "pool" produced by the methods of DiBrino et al. and Zemmour et al. will include MHC trimolecular complexes with **three different** heavy chains.

In summary, while it is agreed that US 5,482,841 and US 5,292,641 teach prior art assays for detecting the presence of antibodies specific for alloantigens, said references do not teach or even suggest the claimed method steps of obtaining a pool of functionally active,

recombinantly produced, truncated individual soluble MHC trimolecular complexes, wherein said complexes have been purified substantially away from other proteins, and wherein each complex includes the same individual MHC heavy chain molecule. All the teachings of the '445, DiBrino et al. and Zemmour et al. references provide to remedy the deficiencies of the '841 and '641 references is the teaching of recombinant production of MHC; however, this clearly does not render obvious the presently claimed invention. In particular, US 6,232,445 does not remedy the deficiencies of the other references, as said reference requires the production of single chain trimers wherein the HLA heavy chain, β-2-microglobulin and peptide are recombinantly attached to each other. The additional references of DiBrino et al. and Zemmour et al. also do not remedy the deficiencies of the other references, as they do not provide (1) a method of producing ***soluble*** MHC heavy chain molecules that are naturally associated with endogenously produced β-2-microglobulin and peptide; or (2) a method of producing a pool of truncated individual soluble MHC trimolecular complexes, wherein said complexes have been **purified substantially away from other proteins**, and wherein each complex includes the **same** individual MHC heavy chain molecule.

Applicants respectfully submit that claims 31-37, 42, 45, 46, 48-51, 60 and 61 are non-obvious over the combination of US 5,482,841, in view of US 5,292,641; US 6,232,445; DiBrino et al. and Zemmour et al. Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. 103(a) rejection of claims 31-37, 42, 45, 46, 48-51, 60 and 61 over US 5,482,841 in view of US 5,292,641; US 6,232,445; US 6,232,445; DiBrino et al. and Zemmour et al.

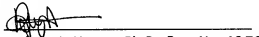
**CONCLUSION**

This is meant to be a complete response to the Office Action mailed May 15, 2009. Applicants respectfully submit that each and every rejection of the claims has been overcome. Further, Applicants respectfully submit that pending claims 31-37, 42, 45-46, 48-51 and 60-61, as now amended, are free of the prior art of record and are in a condition for allowance. Favorable action is respectfully solicited.

In addition, claims 38-41 are currently withdrawn; however, upon allowance of any of claims 31-37, 42, 45-46, 48-51 and 60-61, Applicants respectfully request rejoinder and reconsideration of currently withdrawn claims 38-41. In addition, the Examiner previously required election of a single disclosed species to be used in the claimed method (i.e., a specific substrate, soluble HLA molecule, antibody and anchoring moiety). Upon allowance of any of claims 31-37, 42, 45-46, and 48-51, Applicants respectfully request rejoinder and reconsideration of all disclosed and claimed species (i.e., all specific substrates, soluble HLA molecules, antibodies and anchoring moieties).

Should the Examiner have any questions regarding this amendment, or the remarks contained therein, Applicants' representative would welcome the opportunity to discuss same with the Examiner.

Respectfully submitted,



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